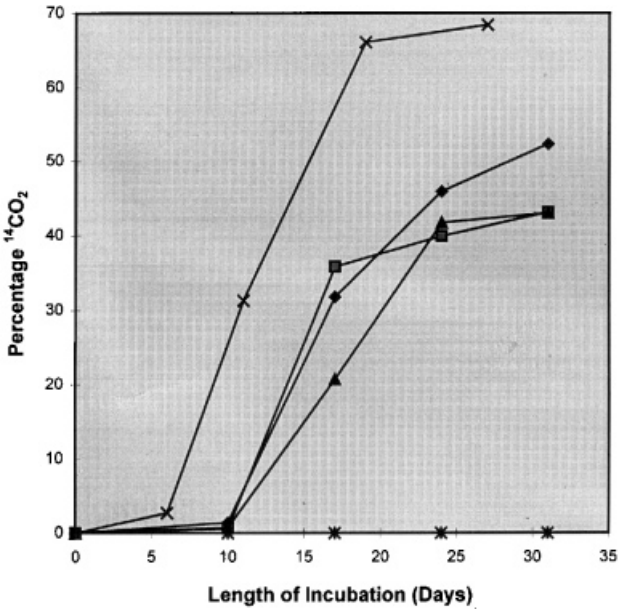

Figure 2. Typical mineralisation plots; cumulative [^{14}C] carbon dioxide, 10 mg litre^{-1} application. × incubation 1, ◆ incubation 2, ■ incubation 3, ▲ incubation 4, ∗ control.

graphy, capillary electrophoresis and mass spectrometry.

The test chemical, [*ring-U*-^{14}C]paraquat (specific activity, 2.0 GBq mmol^{-1}) was supplied by the Jealott's Hill radiochemistry department. The radiochemical was diluted with non-radiolabelled paraquat (99.7% pure) prior to use.

3 RESULTS

Paraquat was extensively metabolised with the rapid production of [^{14}C]carbon dioxide. Typical mineralisation (to CO_2) values were around 50% for both soil extracts and typical [^{14}C]carbon dioxide evolution plots are shown in Fig 2.

Chromatographic analysis of the residual radiochemical in solution at the end of each incubation showed almost identical metabolite profiles between the different micro-organisms. A major metabolite, comprising >85% of the radioactivity remaining in the incubated solution, together with a minor metabolite (<5%), and a metabolite which was incorporated into the degrading microbial cultures (<10%), were characterised. The major metabolite was identified as oxalic acid (**6**), and no paraquat remained in the solutions.

4 DISCUSSION AND CONCLUSIONS

This work has shown that bioavailable paraquat can be rapidly and completely degraded by micro-organisms present in soil. Complete degradation of paraquat takes less than two or three weeks, indicating that the half-life of bioavailable paraquat is considerably shorter than this. The metabolism was so fast that only small fragments of naturally occurring acids, and carbon dioxide as the ultimate mineralisation product, were seen.

These laboratory results correlate well with long-term field trial data, taking into consideration the bioavailability of paraquat in the soil environment.[7]

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Factors controlling degradation of pesticides in soil

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Abstract: Rates of pesticide degradation in soil exhibit a high degree of variability, the sources of which are usually unclear. Combining data from incubations performed using a range of soil properties and environmental conditions has resulted in greater understanding of factors controlling such degradation. The herbicides clomazone, flumetsulam, atrazine, and cloransulam-methyl, as well as the former insecticide naphthalene offer examples of degradation kinetics controlled by coupling competing processes which may in turn be regulated separately by environmental conditions and soil properties. The processes of degradation and volatilization appear to compete for clomazone in solution; sorbed clomazone is degraded only after the solution phase is depleted. Similarly, volatilization of naphthalene is enhanced when degradation has been inhibited by high nutrient levels. Degradation of the herbicide flumetsulam has been shown to be regulated by sorption, even though the compound has a relatively low affinity for the soil. The fate pathway for cloransulam-methyl shifts from mineralization to formation of metabolities, bound residues and physically occluded material as temperature increases. Atrazine degradation in soil may be controlled in part by the presence of inorganic nitrogen, as the herbicide appears to be used as a nitrogen source by micro-organisms. New insight

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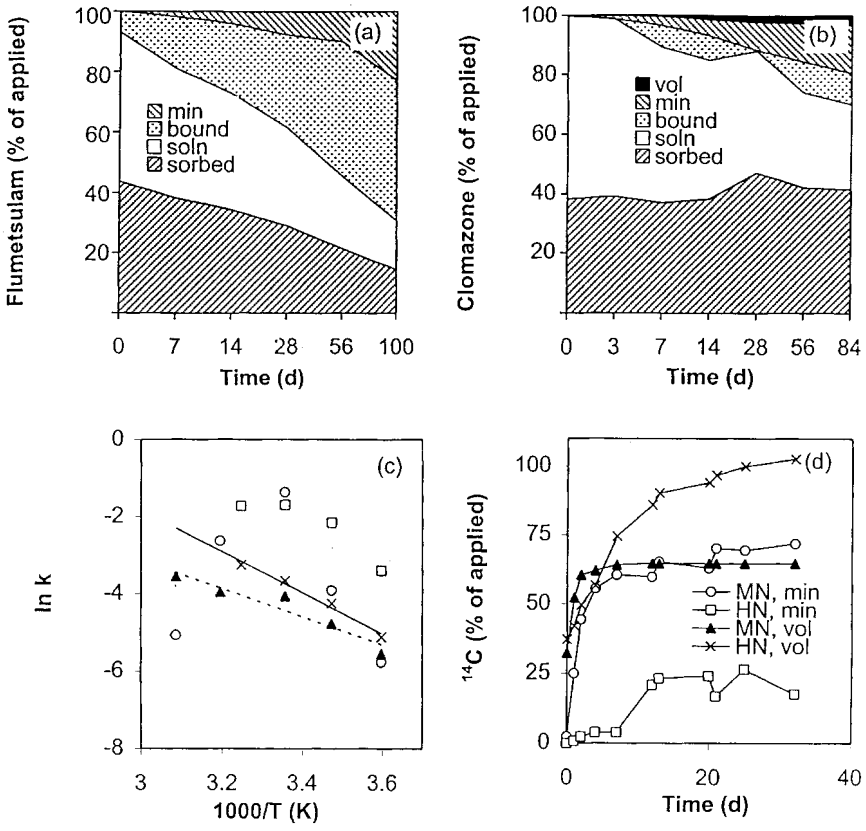

Figure 1. (a) Mass balance of flumetsulam radioactivity over time. Sorbed and solution phases predicted from batch isotherm data presented by authors. (b) Mass balance of clomazone radioactivity as a function of time. (c) Arrhenius plot for mineralization of (○) cloransulam-methyl and (□) clomazone, and microbial respiration in (▲) cloransulam-methyl-treated and (×) clomazone-treated soils. (d) Mineralization (min) and volatilization (vol) of naphthalene at high nutrient (HN) and medium nutrient (MN) concentrations.

gained from measurement of multiple fate processes is demonstrated by these examples.

Keywords: Sorption; pesticide degradation; coupled processes; volatilization

1 INTRODUCTION

Issues such as herbicidal efficacy, carry-over damage and leaching to groundwater necessitate determination of pesticide degradation rates. Many environmental fate processes, including sorption, hydrolysis, volatilization, transport and accumulation of bound residues, are coupled with degradation; each of these processes may respond differently to environmental conditions, thus making comprehension of factors controlling degradation challenging. The importance of process coupling has been recognized recently, leading to studies in which several key fate processes were measured in the same experimental system. This approach has helped establish which factors influence degradation rates over a range of environmental or soil conditions. This summary will evaluate a few of these studies, published and ongoing, in order to illustrate the importance of process coupling on pesticide degradation rates.

2 METHODS AND RESULTS

2.1 Flumetsulam

Lehmann *et al*[1] examined the fate of the herbicide flumetsulam using 21 US soils with a range of properties, and measured dissipation of the parent, mineralization and accumulation of bound residues. Sorption constants (K_d values) were determined using batch isotherms. Field studies included the planting of three sites with a bio-indicator species (*Helianthus annuus* L) to estimate degradation one year after flumetsulam application. Both laboratory and field data indicated that first-order half-life was related to sorption and pH. A simple model, using organic carbon and pH to estimate sorption from K_{oc} values for the neutral and ionic forms of flumetsulam, successfully predicted degradation rates. These results suggest that degradation was controlled by sorption; however, sorbed and dissolved herbicide pools were not differentiated (Fig 1(a)).

2.2 Naphthalene

Malkos[2] used [^{14}C]naphthalene (4ngg^{-1} as an indicator for degradation of hydrocarbons added to soil at 100 to 10 000 μg g^{-1} soil in the presence of a range of added nutrient concentrations. Nutrients were added as $(NH_4)_2 SO_4$, K_2HPO_4, and KCl to provide 170 or 1700 μg of nitrogen, 30 or 300 μg of phosphorus, and 125 or 1250 μg of potassium per gram of soil. The high concentration of nutrients inhibited degradation and stimulated volatilization (Fig 1(d)), suggesting coupling of these processes. Although hydrocarbon rates had little effect on degradation, the highest rate reduced volatilization losses, owing to the affinity of naphthalene for hydrocarbons.

2.3 Clomazone

Mervosh *et al*[3] evaluated the influence of temperature (5–35 °C) and soil water tension (100–1500 kPa) on desorption kinetics as a function of residence time of clomazone in soil. A simple radial diffusion model characterized the desorption process. Increased chemical residence time in soil resulted in decreased desorption, which was attributed to a declining pool of labile material and a stable pool of sorbed clomazone (Fig 1(b)). Degradation, volatilization, bound residue formation, and apparent K_d were temperature-dependent, although the batch equilibrium K_d was temperature-independent. Greatest clomazone mineralization rates[4] occurred at a lower temperature than did the greatest microbial respiration rate (Fig 1(c)).

2.4 Cloransulam-methyl

The initial steps in degradation of a similar herbicide, cloransulam-methyl, appear independent of sorption.[5] Aerobic soil laboratory studies were carried out over a range of temperatures (5–51 °C) for 56 days. Soil was spiked with [^{14}C]cloransulam-methyl and analyzed for parent and metabolites in solution and sorbed phases, soil respiration, [^{14}C] carbon dioxide and bound residue. Additionally, incubated soil was pulverized and extracted with water. As observed previously[4] using clomazone, maximum mineralization of cloransulam-methyl occurred at a lower temperature than the maximum microbial respiration rate (Fig 1(c)). Formation of metabolites and bound residue as well as apparent K_d were temperature-dependent. Soil pulverization released labile herbicide, due to recovery of physically occluded material. The movement of cloransulam-methyl between soil compartments and the relative abundance of metabolites were temperature-dependent though sorption was independent of temperature in batch isotherms. At higher temperatures, the final fate of cloransulam-methyl was predominantly in metabolites, bound residue, and physically occluded pools, as compared to lower temperatures where a pathway leading to [^{14}C] carbon dioxide was favored.

2.5 Atrazine

Culture studies were undertaken with [*ring*-^{15}N] atrazine[6] and three gram-negative bacteria previously identified as atrazine degraders. Soil studies were carried out with double-labeled atrazine [*ring*-^{14}C, *ring*-^{15}N], alternative nitrogen sources and with or without an atrazine degrader. Atrazine degradation was not affected by the presence of other nitrogen sources for constitutive atrazine degraders, but such sources inhibited degradation by an inducible isolate. Mineralization of [^{14}C]atrazine by indigenous soil micro-organisms was also inhibited by inorganic nitrogen, whereas 87% of atrazine was mineralized, regardless of nitrogen status, in the presence of a constitutive atrazine degrader.

3 DISCUSSION AND CONCLUSIONS

Since micro-organisms take up substrates from solution rather than from the sorbed phase,[7] desorption rate-controlled degradation has been used to explain correlations between degradation and sorption parameters. Proof for such relationships is generally derived from degradation studies employing several soils with a range of sorption properties, as reported by Lehmann *et al*[1] for flumetsulam. When sorbed and solution phases are not determined analytically, the relative abundance of these pools is estimated, usually from batch isotherm data. This approach assumes equilibrium, or a constant ratio between the two phases, implying immediate depletion of the sorbed phase (Fig 1(a)). The solution phase, however, is usually depleted before the sorbed phase in experiments in which both pools are measured, resulting in an increase in apparent K_d (Fig 1(b)) over the course of the experiment.[3] The latter approach illustrates the importance of desorption hysteresis and diffusion constraints in unsaturated soil. Volatilization also competes with degradation for substrate in solution, thus conditions that impede degradation indirectly enhance volatilization.[2] Enhancing retention of naphthalene by the addition of hydrocarbons affected volatilization more than degradation, possibly a result of the relative rates of the two processes. Observations with cloransulam-methyl demonstrate shifts in the dominant fate path with changes in a single environmental variable, such as temperature. Effects on fate path may also occur with variation in moisture, soil properties or other factors influencing bioavailability, diffusion, or microbial activity.

Many of the factors controlling pesticide degradation apply generally; however it is important not to overlook unique properties of specific compounds. For example, degradation of atrazine is probably affected by many of the constraints described above, but the role of competing nitrogen sources may be more important in cases where expression of the degradative pathway is inhibited by exogenous nitrogen. The aforementioned examples demonstrate the advantages of measuring coupled processes (sorption, volatilization, transport, physical entrapment, etc) and other controlling factors, (competing nutrients, etc), in order to extend the inference space available for extrapolation of results.

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Molecular cloning and gene expression of two cytochrome P450s from permethrin-resistant *Culex quinquefasciatus* larvae

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Abstract: As part of a continuing study of factors influencing the development of pesticide resistance in insects, two new cytochrome P450s of the CYP6 family have been identified.

Keywords: mosquito; *Culex quinquefasciatus*; resistance; cytochrome P450 monooxygenase; pyrethroid; permethrin; metabolism; synergist; PBO; CYP6E1

Culex quinquefasciatus Say is important as a vector of filariasis in many tropical countries. The prolonged use of organophosphates and carbamates for the control of these mosquito larvae has resulted in the development of resistance to these classes of pesticides. More recently, the extensive use of photostable pyrethroid insecticides to control agricultural pests and disease vectors has also brought about the development of resistance to this class in mosquitoes as well as many other insects of agricultural and medical importance.

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C quinquefasciatus larvae collected from Saudi Arabia (JPal-per) showed a high level of resistance (2 500-fold) to permethrin. In our previous study, a major contribution of P450 monooxygenases in the degradation of pyrethroids in the JPal-per strain was indicated by large differences in the synergistic effects of oxidase inhibitors such as PBO (piperonyl butoxide) and PTPE (2-propynyl 2,3,6-trichlorophenyl ether) on permethrin toxicity between the JPal-per and the susceptible (S) strain.[1] P450 monooxygenases in the microsomes of both larval guts and the remaining body parts metabolized permethrin to 4′-hydroxypermethrin. Furthermore, microsomes from the JPal-per strain were found to have a much greater ability to metabolize permethrin than the S strain, and this activity was inhibited by PBO and PTPE.[1] In order to elucidate the mechanisms of permethrin resistance in *C quinquefasciatus*, we attempted to clone and determine the nucleotide sequences of cytochrome P450 cDNAs.

Cytochrome P450 microsomal monooxygenase-mediated detoxification is a major mechanism by which insects develop resistance to insecticides. Cytochrome P450 genes form a superfamily, and the nucleotide sequences of more than 220 genes have been registered in the DNA data base.[2] These P450 genes are classified into 36 gene families based on the comparison of deduced amino acid sequences.[2] Most of the cytochrome P450s contain a conserved amino acid sequence in the vicinity of the C-terminus, which is involved with a heme-binding region. We designed oligonucleotide primers from this conserved region using sequence data previously reported for CYP6 (cytochrome P450 family 6) isoforms obtained from insecticide-resistant insects including house fly (*Musca domestica* L),[3] cotton bollworm (*Helicoverpa armigera* Hübn),[4] and fruit fly (*Drosophila melanogaster* Meig).[5] A polymerase chain reaction (PCR) was performed using degenerate primers and larval gut cDNA as a template. A cDNA encoding a cytochrome P450 was cloned and found to belong to CYP6 family. We screened a cDNA library constructed from JPal-per larvae using this partial sequence as a probe and determined the complete sequence. This novel P450, designated CYP6E1 was the first reported full-length sequence of a mosquito P450 cDNA.[6]

The deduced amino acid sequence of CYP6E1 was compared to those of cytochrome P450s from other insects (Table 1). CYP6D1 is known to be involved in metabolism of pyrethroid compounds[7] and CYP6A1,[8] CYP6A2[5] and CYP6B2[4] are also cloned from insecticide-resistant insects. The overall homology in amino acid sequence of CYP6E1 to those of CYP6A1, CYP6A2, CYP6B2 and CYP6D1 was 38.9, 35.7, 28.7 and 31.3%, respectively. Percentage divergence showed that CYP6E1 is related to CYP6A and CYP6C subfamilies (Table 1). However, since expression of CYP6E1 was very low and was not found to be very different between permethrin-susceptible and -resistant JPal-per strains, we re-screened the